APh161: Physical Biology of the Cell
Homework 6
Due Date: Thursday, February 23, 2006

"Problems worthy of attack prove their worth by hitting back." -Piet Hein

1. Poster Session Feel for the Numbers.

Give a one paragraph description of the problem you are tackling in your poster. Then, in a page or less, give a series of estimates that will convey a "feeling for the numbers" about your problem to your readers. We are taking this very seriously and so should you. The point is to show that you have made some forward progress on your poster topic. Also, tell us what the toy model is that you are going to use to describe your problem.

2. A Toy Model of FRAP.

One of the intriguing tools for examining the dynamics of cells is a technique known as Fluorescence Recovery After Photobleaching (FRAP). The idea of these experiments was discussed in class and amounts to carving out a hole of photobleached material in a cell and then watching the fluorescence grow back in as a result of diffusion. In this problem, you will work out a one-dimensional toy model of photobleaching.

Imagine a one-dimensional "cell" of length $2L$ (running from $-L$ to L) and with initial concentration c_0 of the fluorescent molecule of interest which is uniformly distributed throughout the cell (note that for this one-dimensional problem the concentration has units of number of molecules per unit length). How many molecules of the fluorescent molecule are there- write an equation that gives this number? Now imagine that we photobleach a hole which runs from $-a$ to a. Before doing any calculations, what will be the final concentration (c_∞) profile once equilibrium is reached again? You may assume that once a molecule is photobleached, it is effectively dead and can be forgotten.

Your goal is to solve for the time evolution of $c(x,t)$ after the photobleaching event. To compute the recovery curves, we first solve the diffusion equation,

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2} \tag{1}$$

for the concentration of fluorescent molecules $c(x,t)$, with the initial concentration after photobleaching given by

$$c(x,0) = \begin{cases} c_0 & \text{for } -L < x < -a \\ 0 & \text{for } -a < x < a \\ c_0 & \text{for } a < x < L \end{cases} . \tag{2}$$

You will impose the boundary condition $\partial c/\partial x = 0$ for $x = \pm L$ which says that the flux of fluorescent molecules across the boundaries of the $2L$-box is zero. This mimics the real-life situation with fluorescent proteins confined to the volume of the cell, to the cell membrane, or some other sub-cellular structure.

To solve the diffusion equation with the prescribed initial and boundary conditions, begin by expanding the concentration profile, $c(x,t)$, in terms of cosine functions,

$$c(x,t) = A_0(t) + \sum_{n=1}^{\infty} A_n(t) \cos\left(\frac{x}{L} n\pi\right) . \tag{3}$$

This expansion guarantees that the boundary conditions are met, namely each of the functions $A_n(t)\cos(xn\pi/L)$ has vanishing first derivatives at $x = \pm L$. Furthermore, since the initial concentration profile is an even function, ie., it takes the same values for positive and negative x, it is readily expanded in cosine functions. The solution of the diffusion equation now boils down to finding the functions $A_n(t)$ such that both eqn.(1) and the initial condition, eqn.(2) are satisfied.

To find these functions, substitute the series expansion of $c(x,t)$ into the diffusion equation. Show that this yields,

$$\frac{dA_0}{dt} + \sum_{n=1}^{\infty} \frac{dA_n(t)}{dt} \cos\left(\frac{x}{L} n\pi\right) = D \sum_{n=1}^{\infty} \left(-A_n(t) \frac{n^2\pi^2}{L^2}\right) \cos\left(\frac{x}{L} n\pi\right) \tag{4}$$

which due to the orthogonality property of the cosine functions for different n turns into a set of independent differential equations

$$\begin{aligned} \frac{dA_0}{dt} &= 0 \\ \frac{dA_n}{dt} &= -\frac{Dn^2\pi^2}{L^2} A_n(t) \quad (n \geq 1) . \end{aligned} \tag{5}$$

Make sure that you show this. The idea is for you to do the entire derivation for this problem step by step and to give a discussion of the logic of each and every step. Show that the solution to each one of these (infinite in number) equations is an exponential function

$$A_n(t) = A_n(0)e^{-\frac{Dn^2\pi^2}{L^2}t} \tag{6}$$

which when substituted into eqn.(3) gives

$$c(x,t) = A_0(0) + \sum_{n=1}^{\infty} A_n(0)e^{-\frac{Dn^2\pi^2}{L^2}t}\cos\left(\frac{x}{L}n\pi\right) \ . \tag{7}$$

The final piece of the puzzle is the determination of the constants $A_n(0)$. To do this, you need to appeal to your initial conditions.

To compute the initial amplitudes of the cosine functions we once again resort to the orthogonality property of these functions

$$\int_{-L}^{L} \cos\left(\frac{x}{L}n\pi\right)\cos\left(\frac{x}{L}m\pi\right) = L\delta_{n,m} \ , \tag{8}$$

where $\delta_{n,m}$ is the Kronecker delta and equals 1 when $n = m$ and 0 otherwise. Show that these orthogonality relations imply the equations

$$\begin{aligned} A_0(0) &= \frac{1}{2L}\int_{-L}^{L} c(x,0)dx \\ A_n(0) &= \frac{1}{L}\int_{-L}^{L} c(x,0)\cos\left(\frac{x}{L}n\pi\right) \quad (n \geq 1) \ , \end{aligned} \tag{9}$$

for the initial amplitudes. Make sure that you prove this previous step. Substitute the initial concentration profile, $c(x,0)$, into these equations, and perform the integrals to show that

$$\begin{aligned} A_0(0) &= c_0\ \frac{L-a}{L} \\ A_n(0) &= -2c_0\ \frac{\sin(n\pi a/L)}{n\pi} \quad (n \geq 1) \ . \end{aligned} \tag{10}$$

Put these results back into the derived formula for $c(x,t)$, eqn.(7) and show that the concentration profile as a function of time is given by

$$c(x,t) = c_0\left[1 - \frac{a}{L} - \sum_{n=1}^{\infty}\frac{2\sin(n\pi a/L)}{n\pi}e^{-\frac{Dn^2\pi^2}{L^2}t}\cos\left(\frac{x}{L}n\pi\right)\right] \ . \tag{11}$$

Make a plot of the concentration profile at various times using a sensible choice of diffusion constant. Show that your result goes to the uniform concentration you deduced before you began the real calculation. Given the concentration profile as a function of time we are now in the position to compute a FRAP recovery curve within our simple one-dimensional model. We ask, how many fluorescent molecules are there in the bleached region as a function of time? In our simple model the bleached region is a box that spans from $-a$ to a on the x-axis. We already know that at $t = 0$, the number of fluorescent molecules in the bleached region is $N_f = 0$ while at times much longer than the diffusion time this number will tend to $c_\infty(2a)$. For intermediate times we need to compute

$$N_f(t) = \int_{-a}^{a} c(x,t)dx \ . \tag{12}$$

Substitute your result for the concentration profile given in eqn.(11) into the integral leads to an expression for the recovery curve. Find an expression for the number of molecules in the photobleached region as a function of time and make a plot of it.

3. Polymerization Reexamined.

In class I described the use of a master equation to deduce the dynamical equation for the mean length of a growing polymer.

(a) Derive the dynamical equation

$$\frac{d\langle L\rangle}{dt} = k_{on}c - k_{off} \tag{13}$$

by starting with a master equation for $dP_n(t)/dt$ and then obtaining $d\langle L\rangle/dt$ by using $< L >= \sum_{n=1}^{\infty} naP_n(t)$. $P_n(t)$ is the probability of polymers of length n at time t. Show that the time evolution of the probability distribution is governed by four distinct classes of process and is captured mathematically as

$$\frac{dP_n}{dt} = k_{on}P_{n-1}P_1 + k_{off}P_{n+1} - k_{on}P_nP_1 - k_{off}P_n. \tag{14}$$

One question of immediate interest that emerges from a model of this kind is what is the average length of a polymer as a function of time whose growth is described by eqn. 14? If a is the length of a monomer we can write the total average length of the filament as

$$\langle L\rangle = \sum_{n=1}^{\infty} n\, a\, P_n. \tag{15}$$

By taking the time derivative of this expression, show that we can then write an equation for the time evolution of the average length as

$$\frac{d\langle L\rangle}{dt} = \sum_{n=1}^{\infty} n\, a\, \frac{dP_n}{dt} \tag{16}$$

Substitute our expressions for the time derivatives themselves from the original rate equation (eqn. 14) and show that this yields

$$\frac{d\langle L\rangle}{dt} = \sum_{n=1}^{\infty} n\, a\, \left(k_{on}P_{n-1}P_1 + k_{off}P_{n+1} - k_{on}P_nP_1 - k_{off}P_n\right). \tag{17}$$

Rearrange the right hand side resulting in

$$\frac{d\langle L\rangle}{dt} = \sum_{n=1}^{\infty} a\, n\, k_{on}P_1\left(P_{n-1} - P_n\right) + \sum_{n=1}^{\infty} a\, n\, k_{off}\left(P_{n+1} - P_n\right).$$

The next step in our argument is to factor out P_1 and to simplify the functional form of the expressions using the identity

$$\sum_{n=1}^{\infty} nP_{n-1} = 2P_1 + 3P_2 + ... = \sum_{n=1}^{\infty}(n+1)P_n. \tag{18}$$

Using this identity several times in their original expression show that

$$ak_{on}P_1\sum_{n=1}^{\infty} n(P_{n-1}-P_n) = ak_{on}P_1\sum_{n=1}^{\infty} nP_n + ak_{on}P_1\underbrace{\sum_{n=1}^{\infty} P_n}_{=1} = ak_{on}P_1 + ak_{on}P_1\sum_{n=1}^{\infty} nP_n. \tag{19}$$

Show that the net result of these manipulations is that we recover precisely the same expression determined earlier for the mean length, namely,

$$\frac{d\langle L\rangle}{dt} = \left(k_{on}P_1 - k_{off}\right)a. \tag{20}$$

(b) Toy model of the catastrophe rate. In class I described a simple model of the catastrophe rate which equated $d\langle L\rangle/dt = a/\tau$, where a is the size per monomer and $1/\tau$ is the rate of hydrolysis. Make a picture and explain the model and then derive a formula for the catastrophe rate as a function of the initial concentration and make a corresponding plot. Try to see how well your result matches with the result from the data shown in the paper by

Holy *et al.*.

4. Myosin and Muscles: Some Estimates.

In class, I described (very briefly) the organization of muscles. In this problem, we will examine all of this in more detail.

a) In your own words, write a "multiscale" description of muscles. That is, describe the various levels in the structural hierarchy of muscles starting with the entire muscle itself (at the largest scales) and ending with the individual myosin molecules (at the smallest scales). Make sure you discuss each structural feature in some detail, making sure to describe the relevant length scales.

b) Make an estimate of the cross-sectional area of a muscle and work your way through to the maximum force available during contraction of the muscle by figuring out the force available per molecule (again, think about a cross section). You will probably have to refer to some of the single molecule work on myosin to really carry out a correct estimate (see Howard, pg. 267, for example). In particular, once you have your estimate of the number of myosins per cross section and the force available per myosin, you will be able to make a preliminary estimate (although not all myosins are attached at all times and you may want to consider that also).